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| (51) International Patent Classification ⁷ : C08B 31/00, C08J 3/12, C08L 3/02, C08B 30/12 | A1 | (11) International Publication Number: WO 00/69916 (43) International Publication Date: 23 November 2000 (23.11.00) |
| (21) International Application Number: PCT/NL00/00050 (22) International Filing Date: 25 January 2000 (25.01.00) (30) Priority Data: 99200203.0 25 January 1999 (25.01.99) EP (71) Applicant (for all designated States except US): ATO B.V. [NL/NL]; P.O. Box 17, NL-6700 AA Wageningen (NL). (72) Inventors; and (75) Inventors/Applicants (for US only): GIEZEN, Franciscus, Egenius [NL/NL]; Nachtegaalstraat 74a, NL-3581 AM Utrecht (NL). JONGBOOM, Remigius, Oene, Jules [NL/NL]; Damstraat 28, NL-6671 AE Zetten (NL). FEIL, Herman [NL/NL]; De Gheijnstraat 10, NL-6717 RG Ede (NL). GOTLIEB, Kornelis, Fester [NL/NL]; Stoepveldsingel 119, NL-9403 SM Assen (NL). BOERSMA, Arjen [NL/NL]; Mr. Van Merwicklaan 3, NL-5237 KL Den Bosch (NL). (74) Agent: JORRITSMA, Ruurd; Nederlandsch Octrooibureau, Scheveningseweg 82, P.O. Box 29720, NL-2502 LS The Hague (NL). | | (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> |
| (54) Title: BIOPOLYMER NANOPARTICLES (57) Abstract A process for producing biopolymer nanoparticles is described, in which the biopolymer is plasticised using shear forces, a crosslinking agent being added during the processing. After said processing, the biopolymer can be dissolved or dispersed in an aqueous medium to a concentration between 4 and 40 wt.%. This results in starch nanoparticles which are characterised by an average particle size of less than 400 nm. | | |

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Biopolymer nanoparticles

[0001] The invention relates to a process for producing nanoparticles, i.e. particles essentially consisting of a biopolymer such as starch. The invention also relates to nanoparticles obtainable by such a process.

[0002] US 5,116,890 discloses self-crosslinking latices on the basis of a starch emulsion polymer graft. These latices require undesirable chemicals and are too hydrophobic for many applications.

[0003] It was found that biopolymers such as starch and other polysaccharides such as cellulose and gums, as well as proteins (e.g. gelatin, whey protein) can be formed into nanoparticles by processing the biopolymer using shear forces and simultaneous cross-linking. The biopolymers may be previously modified, e.g. with cationic groups, carboxymethyl groups, by acylation, phosphorylation, hydroxyalkylation, oxidation and the like. Starch and mixtures of starch with other (bio)polymers containing at least 50% starch are preferred. The biopolymer preferably has a dry substance content of at least 50%, especially at least 60 %by weight at the time when processing starts.

[0004] Processing using shear forces according to the invention means a mechanical treatment, which is in particular an extrusion treatment performed at elevated temperature (above 40°C, especially above 60°C, below the degradation point of the polymer, up to e.g. 200°C, especially up to 140°C) under conditions of high shear. The shear can be effected by applying at least 100 J of specific mechanical energy (SME) per g of biopolymer. Depending on the processing apparatus used the minimum energy may be higher; also when non-pregelatinised material is used, the minimum SME may be higher, e.g. at least 250 J/g, especially at least 500 J/g.

[0005] The mechanical treatment is conveniently performed at elevated temperature. The elevated temperature may be moderated, in case of starch, by using an alkaline medium or by using pregelatinised starch. During the mechanical treatment, the biopolymer is present in high concentration, especially a concentration of at least 40, more preferably at least 50 wt.%, in an aqueous solvent, such as water or a water/alcohol mixture. High pressure (e.g. between 5 and 150 bar) may be applied to facilitate processing at high concentrations.

[0006] A plasticiser may be present in addition to the water or water/alcohol mixture, such as a polyol (ethyleneglycol, propyleneglycol, polyglycols, glycerol, sugar alcohols, urea, citric acid esters, etc.) at a level of 5-40 % by weight of the biopolymer. However, water can already act as a plasticiser. The total amount of plasticisers (i.e. water and other

such as glycerol) is preferably between 15 and 50%. A lubricant, such as lecithin, other phospholipids or monoglycerids, may also be present, e.g. at a level of 0.5-2.5 % by weight. An acid, preferably a solid or semi-solid organic acid, such as maleic acid, citric acid, oxalic, lactic, gluconic acid, or a carbohydrate-degrading enzyme, such as amylase, may be present at a level of 0.01 -5 % by weight of biopolymer; the acid or enzyme assists in slight depolymerisation which is assumed to be advantageous in the process of producing nanoparticles of a specific size.

[0007] An essential step in the process of the invention is the crosslinking during the mechanical treatment. The crosslinking is preferably reversible, i.e. the crosslinks are partly or wholly cleaved after the mechanical treatment step. Suitable reversible crosslinkers include those which form chemical bonds at low water concentrations, which dissociate or hydrolyse in the presence of higher water concentrations. This mode of crosslinking results in a temporary high viscosity during processing followed by a lower viscosity after processing.

[0008] Examples of reversible crosslinkers are dialdehydes and polyaldehydes, which reversibly form hemiacetals, acid anhydrides and mixed anhydrides (e.g. succinic and acetic anhydride) and the like. Suitable dialdehydes and polyaldehydes are glutaraldehyde, glyoxal, periodate-oxidised carbohydrates, and the like. Glyoxal is a particularly suitable crosslinker for the purpose of the invention.

[0009] Such crosslinkers may be used alone or as a mixture of reversible crosslinkers, or as a mixture of reversible and non-reversible crosslinkers. Thus, conventional crosslinkers such as epichlorohydrin and other epoxides, triphosphates, divinyl sulphone, can be used as non-reversible crosslinkers for polysaccharide biopolymers, while dialdehydes, thiol reagents and the like may be used for proteinaceous biopolymers. The crosslinking reaction may be acid- or base-catalysed. The level of crosslinking agent can conveniently be between 0.1 and 10 weight % with respect to the biopolymer. The crosslinking agent may already be present at the start of the mechanical treatment, but in case of a non-pre-gelatinised biopolymer such as granular starch, it is preferred that the crosslinking agent is added later on, i.e. during the mechanical treatment.

[0010] The mechanically treated, crosslinked biopolymer is then formed into a latex by dispersion in a suitable solvent, usually water and/or another hydroxylic solvent such as an alcohol), to a concentration of between 4 and 50 wt.% especially between 10 and 40 wt.%. Prior to the dispersion a cryogenic grinding step may be performed, but stirring with mild heating may work equally well. This treatment results in a gel which either spontaneously

or after induction by water adsorption, is broken into a latex. This viscosity behaviour can be utilised for applications of the particles, such as improved mixing, etc. If desired, the dispersed biopolymer may be further crosslinked, using the same or other crosslinking agents as describe above.

5 [0011] The invention pertains to an extrudate obtained by plasticising a biopolymer, in particular starch, and crosslinking it as described above. The extrudate is characterised by swelling in an aqueous solvent, e.g. water or a mixture of at least 50% water with a water-miscible solvent such as an alcohol, and by exhibiting a viscosity drop afterwards to produce a dispersion of nanoparticles.

10 [0012] The invention also pertains to the nanoparticles obtainable from the extrudate referred to above. The nanoparticles (as such or as a dispersion) are characterised by their small particle size of below 400 nm, especially below 200 nm, their stability, narrow particle size distribution and viscosity. The narrow particle distribution is in particular reflected by the ratio of weight-averaged molecular weight to number-averaged molecular weight being below 2 ($M_w/M_n < 2$).

15 [0013] The nanoparticles can be used as a matrix material i.e. as a resin e.g. in coating applications wherein an increased dry solids content is desired. Such matrix materials may be a film-forming material (for adjusting the minimum film-forming temperature), a thickener, or a rheology modifier, or an adhesive or an adhesive additive (tackifier). As a
20 thickener, it may have higher viscosities, e.g. greater than 150 mPas. The nanoparticles or dispersions thereof may also be used for their barrier properties (high barrier for e.g. oxygen, low barrier e.g. for water), as a carrier (e.g. for colorants, medicaments, flavours and fragrances and the like, advantageously as a slow-release agent), as a fat replacer (due to their mouth-feel), in cosmetic compositions (lack of allergenic properties) etc., as a
25 medicament for mitigating dermal disorders, burns and the like. Further examples of such applications are in the paper-making and packaging industry (paper and cardboard surface treatment, gloss etc.), or in agriculture and horticulture (protective layers on e.g. bulbs, and other plant arts), or as removable or temporary coatings for protection purposes. The nanoparticles can also be used as excipients or carriers e.g. in medicines, where they may be
30 complexed or covalently coupled to active substances such as slow-release drugs. The nanoparticles can also be processed into a foam at relatively high density.

Description of the figure

The figure shows two screw configurations of the extruder used in the examples.

Example 1*Extrusion*

[0014] A premix of native potato starch (12.6 kg, moisture content 18%, Avebe) and glycerol (2.4 kg = 16 wt.% of premix) was prepared at room temperature in a Bear Varimixer for 15 minutes to produce a homogeneous premix. The premix was fed into an extruder (Berstorff ZE40 self-wiping, co-rotating twin screw extruder, 38D) at a rate of 8.4 kg/hr with a K-Tron K2M &85 volumetric feeder. The extruder has 9 zones with an adjusted temperature profile of 20-80-90-100-100-100-100-100-95 °C. Screw configuration 1 (Figure) was used. The screw speed was set at 160 rpm. A 5 wt.% solution of glyoxal in water was injected in the barrel at the 5th zone (22D), at a flow rate of 1.2 kg/hr with a Prominent Meta HK20-12,5S piston dosing pump. As a result, the pure glyoxal percentage in relation to the dry amount of starch was 1%. The extrudate left the extruder through a five-hole die (diameter of holes 3 mm) and was subsequently granulated.

Latex preparation (1)

[0015] The granules were cryogenically ground and sieved to obtain particles smaller than 150 µm. Subsequently, this powder was mixed with water. The amount of powder was adjusted to the desired solid load in the final dispersion. After stirring the mixture at room temperature, a strong gel was formed. This gel collapsed after some time, and a low-viscous homogeneous transparent light-brown dispersion was formed. The viscosity of 10w/w% dispersion was measured with a Brookfield DV-III Viscometer, showing a viscosity of 26 mPa.s at a shear rate of 55 s⁻¹ at room temperature. The particle radii in a 10 w/w% dispersion were determined by means of DLS (Dynamic Light Scattering), showing values between 10 nm and 500 nm, and a calculated average particle radius of 54 nm.

Latex preparation (2)

[0016] The granules were cryogenically ground and sieved to obtain particles smaller than 500 µm. 10 g of this powder was mixed with 90 g of water. After stirring the mixture at room temperature, a strong gel was formed. This gel collapsed after some time, and a low-viscous homogeneous transparent light-brown dispersion was formed. The particle radii were determined by means of DLS (Dynamic Light Scattering), showing values between 10 nm and 500 nm.

Latex preparation (3)

[0017] 10 g of the granules were mixed with 90 g of water. After stirring the mixture at 50 °C for 2 hr a low viscous dispersion was formed. After filtering of the dispersion over a

150 μm filter cloth the particle sizes were determined by means of DLS, showing radii between 10 nm and 500 nm.

Latex preparation (4)

[0018] 20 g of the granules were mixed with 80 g of water. After stirring the mixture at 50 °C for 2 hr a low viscous dispersion was formed. After filtering of the dispersion over a 150 μm filter cloth the viscosity of the dispersion was determined with a Brookfield DV-III Viscometer, showing a viscosity of 825 mPa.s at a shear rate of 55 s^{-1} at room temperature.

Film formation

[0019] The dispersion obtained above (20 g) was casted into a polystyrene tray (casting onto glass, paper is also feasible) and subsequently dried at room temperature. After two days a transparent strong starch film remained.

Examples 2-6

[0020] Example 1 was repeated in a continuous mode: 8.81 kg of premix (containing 6.48 kg of dry potato starch, 18.0 wt.% = 1.17 kg of water and 17.9 wt.% = 1.16 kg of glycerol) was fed per hour. A glyoxal solution containing 5-10-15 wt.% of glyoxal in water (0.93-1.86-2.80 wt.% with respect to dry starch) was fed at a rate of 1.2 kg/hour. The results are presented in the following table.

Table

[0021] Results of continuous thermomechanical treatment and crosslinking

| example | 2 | 3 | 4 | 5 | 6 |
|-----------------------------|------|------|-------|-------|------|
| X-link wt.% ¹ | 10 | 15 | 5 | 10 | 10 |
| screw speed | 200 | 200 | 100 | 100 | 250 |
| screw conf. ² | 1 | 1 | 1 | 1 | 2 |
| SME (J/g) | 1765 | 1818 | 972 | 1186 | 3316 |
| viscosity ³ mPas | 27.8 | 17.0 | 185.0 | 118.3 | 17.5 |
| particle radius nm | 42.5 | 36.9 | 101.4 | 53.4 | 41.9 |

¹: concentration of crosslinker in water (1% corresponding to about 0.186 wt.% with respect to dry starch)

²: see accompanying figure

³: determined of a 10% dispersion at 186 s^{-1} and 20°C

Example 7

[0022] A premix of waxy corn starch (moisture content 11.5 %, Meritena 300, Amylum), glycerol (18 %, based on dry starch) and water (total water content adjusted to 22 %, based on dry starch) was fed into the feeding zone of a Berstorff ZE40 extruder ($L/D = 38$) at a rate of 8.8 kg/h. The temperature profile in the extruder was similar to the one described in example 1. Screw configuration 1 was used and the screw speed was set on 200 rpm. A glyoxal solution of 10 % in water was fed in the 5th zone (22D) at a rate of 1.2 kg/h. The extrudate was dried and granulated. Dispersion preparation was performed as described in example 1 (latex preparation 1). A 20 % dispersion was obtained having a Brookfield viscosity of 225 mPas at a shear rate of 20 s^{-1} .

Example 8

[0023] A premix of waxy rice starch (moisture content 14 %, Remyline AX-DR, Remy), glycerol (18 %, based on dry starch) and water (total water content adjusted to 22 %, based on dry starch) was fed into the feeding zone of a Berstorff ZE40 extruder ($L/D = 38$) at a rate of 8.8 kg/h. The temperature profile in the extruder was similar to the one described in example 1. Screw configuration 1 was used and the screw speed was set on 200 rpm. A glyoxal solution of 10 % in water was fed in the 5th zone (22D) at a rate of 1.2 kg/h. The extrudate was dried and granulated. Dispersion preparation was performed as described in example 1 (latex preparation 1). A 20 % dispersion was obtained having a Brookfield viscosity of 253 mPas at a shear rate of 20 s^{-1} .

Example 9

[0024] A premix of modified (acetylated and crosslinked) waxy corn starch (moisture content 12.7 %, Resistamyl 342, Amylum), glycerol (18 %, based on dry starch) and water (total water content adjusted to 22 %, based on dry starch) was fed into the feeding zone of a Berstorff ZE40 extruder ($L/D = 38$) at a rate of 8.8 kg/h. The temperature profile in the extruder was similar to the one described in example 1. Screw configuration 1 was used and the screw speed was set on 200 rpm. A glyoxal solution of 10 % in water was fed in the 5th zone (22D) at a rate of 1.2 kg/h. The extrudate was dried and granulated. Dispersion preparation was performed as described in example 1 (latex preparation 1). A 20 % dispersion was obtained having a Brookfield viscosity of 180 mPas at a shear rate of 20 s^{-1} .

Example 10

[0025] A premix of waxy corn starch (moisture content 11.5 %, Meritena 300, Amylum), glycerol (18 %, based on dry starch), water (total water content adjusted to 22 %, based on

dry starch) and maleic acid (0.5 % based on dry starch) was fed into the feeding zone of a Berstorff ZE40 extruder ($L/D = 38$) at a rate of 8.8 kg/h. The temperature profile in the extruder was similar to the one described in example 1. Screw configuration 2 was used and the screw speed was set on 200 rpm. A glyoxal solution of 10 % in water was fed in the 5th zone (22D) at a rate of 1.2 kg/h. The extrudate was dried and granulated. Dispersion preparation was performed as described in example 1 (latex preparation 1). A 35 % dispersion was obtained having a Brookfield viscosity of 1400 mPas at a shear rate of 20 s^{-1} .

Claims

1. A process for producing biopolymer nanoparticles, in which the biopolymer is plasticised by processing using shear forces, a crosslinking agent being present during the processing.
2. A process according to claim 1, wherein the biopolymer is starch or a starch derivative or a polymer mixture containing at least 50 % starch.
3. A process according to claim 1 or 2, wherein the crosslinking agent is a dialdehyde or polyaldehyde, especially glyoxal.
4. A process according to any one of claims 1-3, wherein the processing is carried out at a temperature of between 40 and 200°C.
5. A process according to any one of claims 1-3, wherein shear forces are used corresponding to a mechanical energy input of at least 100 J/g.
6. A process according to any one of the preceding claims, wherein the biopolymer has a dry substance content during processing of at least 50% by weight.
7. A process according to any one of the preceding claims, wherein the processing comprises extrusion.
8. A process according to any one of the preceding claims, wherein 1-40 wt.%, based on the weight of the biopolymer, of an additional plasticiser such as glycerol is present during the processing.
9. A process according to any one of the preceding claims, wherein 0.01-5 %, based on the weight of the biopolymer, of an acid such as maleic acid, oxalic acid or citric acid is present during processing.
10. A process according to any one of the preceding claims, wherein 0.01-5 %, based on the weight of the biopolymer, of an enzyme such as amylase is present during processing.

11. A process according to any one of the preceding claims, wherein after said processing, the biopolymer is dissolved or dispersed in an aqueous medium to a concentration between 4 and 40 wt.%.
12. A process according to claim 11, wherein the dispersion of the biopolymer is further crosslinked after said processing.
13. Starch nanoparticles which - optionally after dispersion in water - are characterised by an average particle size of less than 400 nm and a narrow particle size distribution ($M_w/M_n < 2$).
14. A dispersion of crosslinked starch nanoparticles according to claim 13 in water.
15. A dispersion according to claim 14, having a viscosity at a concentration of 10% by weight at 186 s^{-1} of below 150 mPas, preferably below 50 mPas.
16. A extrudate of crosslinked polymer containing at least 50% starch, which swells upon immersion in an aqueous medium and forms a low viscous dispersion after immersion.

INTERNATIONAL SEARCH REPORT

International Application No.
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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C08B31/00 C08J3/12 C08L3/02 C08B30/12

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| A | FR 2 265 762 A (HENKEL & CIE GMBH) 24 October 1975 (1975-10-24) page 1, line 34 - line 37 claims; examples | 1-4, 6, 13 |
| A | EP 0 118 240 A (WARNER-LAMBERT COMPANY) 12 September 1984 (1984-09-12) abstract page 23, line 25 - line 36 claim 3 | 1-8 |
| P, A | US 6 001 408 A (DUDACEK ET AL.) 14 December 1999 (1999-12-14) abstract; example 1 | 1, 2, 4-8, 13 |
| A | US 4 072 535 A (SHORT ET AL.) 7 February 1978 (1978-02-07) abstract | 13 |
| -/- | | |

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| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | EP 0 525 245 A (BUTTERFLY SRL.) 3 February 1993 (1993-02-03) page 4, line 38 - line 55 claim 1 | 16 |
| A | US 5 412 005 A (BASTIOLI ET AL.) 2 May 1995 (1995-05-02) column 1, line 24 - line 36 column 10, line 49 - line 67 | 16 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No
PCT/NL 00/00050

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|---|--|
| FR 2265762 A | 24-10-1975 | DE 2415556 A JP 1268501 C JP 50130838 A JP 59045685 B US 3997508 A ZA 7501956 A | 16-10-1975 10-06-1985 16-10-1975 08-11-1984 14-12-1976 25-02-1976 |
| EP 118240 A | 12-09-1984 | AT 44975 T AU 572119 B AU 2465684 A BG 46154 A BR 8400734 A CA 1238738 A CS 8401078 A CS 8505547 A DD 218113 A DE 3479129 D DK 76584 A EG 17995 A ES 529815 D ES 8505385 A FI 840614 A,B, GR 82253 A HU 36487 A,B IE 56888 B IL 70980 A IN 160476 A IN 165520 A JP 1838984 C JP 59196335 A KR 9304937 B MX 170518 B NO 840598 A,B, NZ 207188 A PH 20759 A PL 246259 A PT 78123 A,B RO 88123 A RU 2042423 C TR 23047 A YU 28684 A US 4673438 A US 4738724 A | 15-08-1989 05-05-1988 23-08-1984 16-10-1989 02-10-1984 28-06-1988 12-02-1987 12-02-1987 30-01-1985 31-08-1989 19-08-1984 30-06-1992 16-05-1985 01-09-1985 19-08-1984 13-12-1984 30-09-1985 15-01-1992 31-03-1987 11-07-1987 04-11-1989 25-04-1994 07-11-1984 10-06-1993 27-08-1993 20-08-1984 10-09-1986 10-04-1987 13-02-1985 01-03-1984 30-11-1985 27-08-1995 14-02-1989 31-12-1986 16-06-1987 19-04-1988 |
| US 6001408 A | 14-12-1999 | AU 707179 B AU 6818396 A CA 2187725 A JP 9165401 A | 01-07-1999 17-04-1997 14-04-1997 24-06-1997 |
| US 4072535 A | 07-02-1978 | NONE | |
| EP 525245 A | 03-02-1993 | AT 155161 T AU 658180 B AU 1650992 A AU 658207 B AU 2058292 A BR 9203064 A BR 9205258 A | 15-07-1997 06-04-1995 21-12-1992 06-04-1995 04-02-1993 30-03-1993 27-07-1993 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/NL 00/00050

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| EP 525245 A | | CA 2074649 A | 02-02-1993 |
| | | CA 2084994 A | 04-11-1992 |
| | | CN 1071588 A | 05-05-1993 |
| | | CN 1077966 A, B | 03-11-1993 |
| | | CZ 9202395 A | 17-02-1993 |
| | | CZ 285748 B | 13-10-1999 |
| | | DE 9219021 U | 27-02-1997 |
| | | DE 69220754 D | 14-08-1997 |
| | | DE 69220754 T | 04-12-1997 |
| | | DE 539541 T | 30-01-1997 |
| | | DK 539541 T | 15-09-1997 |
| | | WO 9219680 A | 12-11-1992 |
| | | EP 0539541 A | 05-05-1993 |
| | | ES 2103943 T | 01-10-1997 |
| | | FI 925978 A | 31-12-1992 |
| | | GR 3024078 T | 31-10-1997 |
| | | HU 216971 B | 28-10-1999 |
| | | JP 5228205 A | 07-09-1993 |
| | | JP 2527523 B | 28-08-1996 |
| | | JP 6502676 T | 24-03-1994 |
| | | KR 9608112 B | 20-06-1996 |
| | | LV 12151 A | 20-10-1998 |
| | | LV 12151 B | 20-12-1998 |
| | | NO 925049 A | 30-12-1992 |
| | | PL 295469 A | 04-05-1993 |
| | | RU 2095379 C | 10-11-1997 |
| | | SK 390192 A | 07-12-1994 |
| | | RU 2089151 C | 10-09-1997 |
| | | US 5286770 A | 15-02-1994 |
| | | US 5412005 A | 02-05-1995 |
| US 5412005 A | 02-05-1995 | EP 0525245 A | 03-02-1993 |
| | | IT 1245485 B | 20-09-1994 |
| | | IT 1256693 B | 12-12-1995 |
| | | IT 1263114 B | 30-07-1996 |
| | | CN 1077966 A, B | 03-11-1993 |
| | | IL 104942 A | 10-06-1997 |
| | | AT 155161 T | 15-07-1997 |
| | | AU 658180 B | 06-04-1995 |
| | | AU 1650992 A | 21-12-1992 |
| | | AU 658207 B | 06-04-1995 |
| | | AU 2058292 A | 04-02-1993 |
| | | BR 9203064 A | 30-03-1993 |
| | | BR 9205258 A | 27-07-1993 |
| | | CA 2074649 A | 02-02-1993 |
| | | CA 2084994 A | 04-11-1992 |
| | | CN 1071588 A | 05-05-1993 |
| | | CZ 9202395 A | 17-02-1993 |
| | | CZ 285748 B | 13-10-1999 |
| | | DE 9219021 U | 27-02-1997 |
| | | DE 69220754 D | 14-08-1997 |
| | | DE 69220754 T | 04-12-1997 |
| | | DE 539541 T | 30-01-1997 |
| | | DK 539541 T | 15-09-1997 |
| | | WO 9219680 A | 12-11-1992 |
| | | EP 0539541 A | 05-05-1993 |
| | | ES 2103943 T | 01-10-1997 |
| | | FI 925978 A | 31-12-1992 |

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/NL 00/00050

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| US 5412005 A | | GR 3024078 T | 31-10-1997 |
| | | HU 216971 B | 28-10-1999 |
| | | JP 5228205 A | 07-09-1993 |
| | | JP 2527523 B | 28-08-1996 |
| | | JP 6502676 T | 24-03-1994 |
| | | KR 9608112 B | 20-06-1996 |
| | | LV 12151 A | 20-10-1998 |
| | | LV 12151 B | 20-12-1998 |
| | | NO 925049 A | 30-12-1992 |
| | | PL 295469 A | 04-05-1993 |
| | | RU 2095379 C | 10-11-1997 |
| | | SK 390192 A | 07-12-1994 |
| | | RU 2089151 C | 10-09-1997 |
| | | US 5286770 A | 15-02-1994 |
| | | AT 127034 T | 15-09-1995 |
| | | DE 69204351 D | 05-10-1995 |
| | | DE 69204351 T | 11-04-1996 |
| | | DK 512360 T | 18-09-1995 |
| | | EP 0512360 A | 11-11-1992 |
| | | ES 2077280 T | 16-11-1995 |
| | | GR 3017583 T | 31-12-1995 |
| | | JP 5123550 A | 21-05-1993 |
| | | PL 174799 B | 30-09-1998 |